

EXHIBIT A

Project No. 3

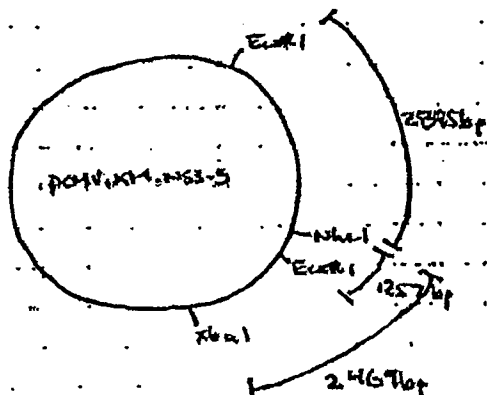
Book No. 10384

TITLE Construct ANS3-NBS into yeast

From Page No. X

Mark Salby came and asked us to adapt a construct he created into yeast. His construct is currently in a pCMV vector, containing NS3 to NS5, cloned from an EcoRI to Xba fragment.

To Adapt this construct to yeast, we can take out this fragment (actually as two pieces), add some synthetic linkers to be able to ligate w/ our ADH2/GAPDH promoter and yeast vector.



From plasmid cut must give to us

as 2 digests: 1) EcoRI - NheI

2) NheI - Xba

EcoRI - NheI		NheI - Xba	
10xM	20	10xM	20
EcoRI	12	NheI	10
NheI	10	XbaI	10
pCMV-AN3-N5	10	pCMV-AN3-N5	10
ADH2	150	ADH2	150
200µl		200µl	

→ 4hr @ 37°C → Freeze @ -20°C

→ Run on 1% agarose

Purify by Phage 100 Purification protocol

EDTA in 75µl of 100µl. Check for 100µl

EN NIX

EN = 7µl of 0.025µl

NIX = 10µl of 0.025µl

To Page No. 11

Witnessed & Understood by me,

[Signature]

Date

Invented by

[Signature]

Date

Recorded by